# An Experimental Investigation concerning the Effects of "Tuba" (Derris elliptica) Fish-Poison.

By J. Argyll Campbell.

"Tuba" is a term used by the Malays to denote various plants which possess fish-poisoning properties. *Derris elliptica* Benth., Leguminosæ, is the most powerful of these plants.

Interesting accounts of "tuba" fishing are given by George Maxwell (1) and W. H. Furness (2). According to these authors the root of the low climbing plant, *Derris elliptica,* is most commonly employed. The root is pounded by a club and then extracted by soaking it in water contained in boats. The soaked root is compressed and a milky-white watery fluid escapes. This fluid is mixed with lime to make it sink and spread when poured into the river. Previously a barricade has been erected some distance down the river to prevent the fish escaping. The poison stupefies the fish and they flee before it. Men in boats and on the barricade scoop up the fish in nets or spear them as they come to the surface. Furness says that the fish seem to be affected by suffocation.

Research has been done concerning the chemical composition of this poison. M. Greshoff (3) obtained a resin which he called "derrid" and which he found to resemble pachyrrhizid, timboine, nicuoline and piscidine in composition. Wray (4) also isolated a resinous substance which he named "tubaine."

I can find no research concerning the actions of the poison upon the living tissues, although much has been written about its apparent actions. Greshoff (5) says that drinking the poison produces vomiting, dizziness and death. He records a case of suicide in Java. Ridley (6) mentions that "tuba" poison is speedily fatal to man when swallowed or to fish when in contact with the gills; and that it is used by Malays as an abortifacient (7). Gimlette (8) gives a good deal of information. Fish stupefied by the poison can be eaten with impunity by man. Chinese use the poison extensively as an insecticide, especially for spraying pepper vines. It is put into wells with criminal intent, but death seems to be rare. A decoction is used by Malay girls to produce abortion; death sometimes occurs owing to uterine haemorrhage. Acute cases of poisoning are characterised by fixation of the jaws. In Borneo the Dyak girls use it to commit suicide. It is also mixed with "ipoh" poison by the Sakei in preparation of dart poison for blow pipes.

## Preparation of the Poison.

In my research the extract was prepared in the same way as Malay fishermen prepare it. When necessary the root was not extracted with water, but with Ringer's physiological saline solution (NaCl .9%; KCl .01%; $Ca_3(PO_4)2$ to saturation). It was then filtered and boiled. These processes do not interfere with its toxic properties and the fluid is still milky-white. The poison passes very slowly through parchment; only one seventieth part of the poison passes through in five days, so that it seems to be in colloidal solution. The extract keeps at least for a week. In most cases the extract was prepared fresh when required. The specimen of the root used by me, was kept for three months in a cupboard and retained its full powers all that time.

The extract is not antiseptic. Organic matter soaked in it soon putrefies at ordinary room temperature (28 C).

The extract is faintly acid in reaction. It has an acrid taste and smell. The taste persists for a long time; strong solutions cause slight numbness of the gums and mouth about ten minutes after tasting.

I have not attempted to separate the active substance, but I have investigated the actions of the extract as used by the natives.

In estimating the strengths of the solutions used, I have taken 1 gm. by weight of the root in 100 cubic centimeters of water as 1 in 100 solution.

Before performing any experiments, the milky extract to be used was first tested to prove that it was capable of killing fish. It never failed to do this. A given weight of the root contains a constant quantity of the poison and kills fish in a definite time.

## Effects on Different Animals.

Effects on Fish. Fish, *Ophiocephalus gachua,* Buch.-Ham., of about fifty grammes weight were used. Solutions as weak as 1 in 100,000 are fatal to these fish. Wray (9) found that 1 in 350,000 of the isolated resin "tubaine" kills fish in half an hour. Greshoff (9) obtained the same results with a much weaker solution of the resin "derrid."

The symptoms of poisoning as observed by myself are constant. In a solution 1 in 4500 the fish becomes agitated almost at once and swims about wildly at the surface of the water taking in mouthfuls of air which escapes into the water by the gill slits. In two minutes time the fish is lying on its side at the bottom of the vessel, breathing slowly and deeply. In another five minutes the breathing stops, the fins twitch, but reflex movements can be elicited for another three minutes. The fish dies eleven minutes after immersion. A solution 1 in 12,000 kills in twenty eight minutes, a solution 1 in 50,000 in eighty three minutes. The fish

does not seem to lose consciousness until the breathing is greatly affected. Post mortem examination shows venous congestion of the organs; the heart is full of blood, but still capable of contracting in response to stimuli. If the heart be examined soon after the breathing has stopped, it is observed to be beating feebly, and if relieved from the distension with blood, will beat for a long time.

From these observations it seems very probable that death is due to asphyxia.

Another series of experiments was done in which a small quantity (1 cubic centimeter) of the extract was injected into the stomach of the fish by means of a long narrow metal tube passed down the œsophagus.

The minimal lethal dose, in this manner of injection, is the extract obtained from 1/200 gm. of the root. The extract actually injected is 1 c. c. 1 in 200 solution. The symptoms of poisoning are exactly the same as those described above. The poison is rapidly absorbed by the stomach, the fish becoming affected two minutes after the injection. Wray (9) points out that "tubaine" is insoluble and that he has seen a fish eat a quantity without ill effects. I have not used the isolated substance "tubaine," but the milky extract is certainly rapidly fatal when administered in this way. The extract from 1/25 gm. of the root kills fish in twelve minutes, from 1/200 gm. in one hour.

I have not experimented with fish larger than 50 gm. Undoubtly larger fish would require larger doses to kill them.

Effects on Tadpoles. Tadpoles of the common Singapore toad, *Bufo melanostictus,* were immersed in solutions of various concentrations. The symptoms of poisoning closely resemble those described for fish, but stronger solutions are required. Solutions weaker than 1 in 12,000 do not kill tadpoles. A solution 1 in 12,000 kills in forty three minutes and a solution of 1 in 4,500 in twenty minutes. These solutions kill fish in half these times respectively.

Effects on Mosquitœ Larvæ. Stegomyia larvæ were employed. Solutions weaker than 1 in 10,000 are not fatal, nor do they prevent the larvæ developing. A solution 1 in 10,000 takes at least two days to kill larvæ; 1 in 5,000 kills them in about two days; 1 in 1,000 in one day, 1 in 500 in three hours, 1 in 10 in one hour. Therefore much stronger solutions and much longer periods of action are required to kill mosquitœ larvæ than to kill fish or tadpoles.

Effects on Toads. The common toad, *Bufo melanostictus,* was employed. The poison was administered either by subcutaneous injection or by injection into the stomach of one cubic centimeter of the fluid extract. The minimal lethal dose by the

first method of injection is 1 c. c. 1 in 50, that is the extract obtained from 1/50 gm. of the root, by the second method of injection 1 c. c. 1 in 12, that is the extract obtained from 1/12 gm. of the root. In both cases the symptoms are similar. In a few minutes the animal becomes agitated for a short time, then quietens down and in another ten minutes its breathing becomes deeper and irregular in rate, the nostrils dilating at each inspiration. The animal is still conscious and all its reflexes are normal. In another twenty minutes the breathing stops, reflexes diminish and finally disappear about thirty five minutes after the injection. Post mortem examination reveals signs of death from asphyxia, the organs being congested, and the heart full. The heart can be stimulated to contract for a long time after removal from the animal.

Effects on Monkeys. Two varieties of monkeys were used, the common kra (*Macacus cynomolgus*) and the larger pig-tailed monkey (*Macacus nemestpinus*).

The poison was injected subcutaneously; the injection causes no pain. The minimal lethal dose is the extract from 2 gm. of the root. The extract is concentrated to small bulk by boiling; boiling does not effect the poison. Within five minutes the animal becomes weak, and the gums and tongue are very pale. There is usually slight vomiting about this time. The vomiting resembles cerebral vomiting and soon ceases as the animal goes to sleep. It can be aroused and its reflexes are normal. Then the breathing shows great increase in depth, and soon becomes very deep and slow; then gasping inspirations occur about six a minute; the reflexes now disappear and the animal cannot be aroused; gasping gradually ceases and the animal dies. The heart beats for several minutes after the respiration ceases. These symptoms are those of asphyxia and post mortem examination shows the signs of death from asphyxia. Death occurs in about forty minutes.

Experiments in which the poison is introduced into the stomach by a stomach tube give the same results. Vomiting comes on in about five minutes and is not excessive. In any case sufficient poison is absorbed because death takes place even after vomiting. The extract from 2 gm. weight of the root is the minimal lethal dose. Death occurs in forty minutes, the symptoms resembling those produced by subcutaneous injection of the poison. When the respiration is greatly affected, the reflexes disappear, the corneal reflex being the last to go. The pupils are dilated.

## Action on the Tissues and Organs Removed from the Body.

Effects on Voluntary Muscle. When in concentrations 1 in 8 to 1 in 2,000, its action on the voluntary muscles of the tongue and of the calf of a toad is to weaken their power of contraction. The motor nerves and end plates are not affected. This

weakening of the muscle is removed by washing out the poison. Weaker solutions have no action on voluntary muscle.

In the experiments on the tongue the poison was injected under the mucous membrane. Induction shocks were used to stimulate the muscle.

Effects on Involuntary Muscle. "Tuba" poison in solutions 1 in 60 to 1 in 6,000, diminishes the tone and movements of the involuntary muscle of the intestine of a monkey. This weakening is counteracted by the presence of a dilute solution of sodium carbonate.

Effects on Heart Muscle. "Tuba" poison, even in strong solutions, has no action on heart muscle. The isolated heart beats strongly and for a long time in Ringer's solution containing the poison in concentrations 1 in 8 or 1 in 100. The same results are obtained when the heart is perfused with the poison 1 in 80.

These results with heart muscle, verify the fact that for some time after the respiration has ceased in the living animal under the influence of the poison, the heart may be felt still beating strongly.

Effects on the Blood. Strong solutions (1 in 100) of the poison in Ringer's solution alter neither the red nor white cells of the blood of the monkey. The white cells show the usual amœboid movements if the solution be kept warm. There is no haemolysis or breaking up of the red cells.

The oxygen capacity of the blood is not altered by the poison, the red cells taking up oxygen easily and giving it off easily.

Effects on the Blood Vessels. Solutions of the poison varying in strength from 1 in 30 to 1 in 4000 were perfused through the blood vessels of a toad. In most cases the poison dilates the blood vessels, in a few cases no effect is produced.

In another series of experiments the extract (from 1 in 50 up to 1 in 1250) was perfused through the blood vessels of a limb of a monkey. In these vessels dilatation is produced and the poison so affects the blood vessels that the power of adrenalin to constrict them is markedly lessened and in most cases abolished.

### Experiments on Anaesthetised Monkeys.

*Macacus cynomolgus* and *Macacus nemestpinus* were the monkeys employed, chloroform being used as the anaesthetic. The poison was injected into a vein, usually the femoral vein, by means of an injection cannula; records of the blood pressure, usually that of the femoral artery, and of the respiration were taken.

Injected in this way the extract from 1/50 gm. of the root is sufficient to produce death. In all cases marked effects are produced on the respiration and blood pressure (Fig. 1). Respiration is usually stimulated at first, then depressed and finally paralysed. The blood pressure falls considerably but only temporarily.

Later further changes are produced in the blood pressure, but these are produced by the asphyxia.

A dose as weak as 1 c. c. of 1 in 10,000 solution produces similar changes in the respiration and blood pressure but the changes are not so well marked; the respiration is not paralysed and death does not occur, the normal conditions prevailing again.

The tissues apparently become accustomed to the poison in a slight degree. Thus a dose from 1/50 gm. of the root kills if injected without any previous injection being made; but if many injections of weaker solutions of gradually increasing strengths are first performed, a stronger dose than 1/50 gm. is required to produce death.

The poison acts upon the respiratory nervous centre in the medulla and not on the vagal ending in the lungs, because the same results are obtained if the vagi are cut (Fig. 2). Also if the poison is injected into the carotid artery, the respiration is affected in a few seconds.

It has already been stated that the poison dilates the blood vessels of the isolated limb of a monkey and that it greatly weakens the tone and movements of the involuntary muscle of the intestine of the same animal. The fall of blood pressure is explained by these actions. Further experiments and chemical analysis are required to prove whether one substance causes both paralysis of respiration and dilatation of the blood vessels or whether there are two distinct substances for these actions.

The previous injection of adrenalin only slightly modifies the depressing influence of the poison upon the blood vessels, and the fall of blood pressure is still very well marked.

### After-effects of the Poison.

In some experiments injections were made subcutaneously into monkeys, but although these injections were strong enough to produce very great effects on the respiration, causing a marked degree of asphyxia, they were not strong enough to kill the animals. In these cases the animals recover completely in a few hours and exhibit no ill after-effects,—no paralysis, no digestive troubles and no weakness of any kind.

### Discussion.

From the results on different animals it is evident that the poison affects the more highly developed members of the animal kingdom more readily that it does the primitive members. This is only to be expected since its action concerns the brain and one particular part of this, namely the medulla oblongata.

It could be used to destroy mosquitœ larvæ, but it should be used in solutions not weaker than 1 in 1,000, that is just enough

of the extract should be added to the pool to make the water cloudy. Of course this would kill fish up to 50 gm. far more quickly than it would the larvæ and in time it would certainly affect much larger fish.

Its effects on the higher animals can be explained from the results obtained in the experiments performed on anaesthetised animals. The great weakness exhibited in cases of poisoning is due to the fall of blood pressure and to the weakening of the voluntary and involuntary muscle. The effects on the respiration are due at first to the stimulation and afterwards to the paralysis of the respiratory centre. The vomiting which usually occurs seems to be due to the stimulation of the vomiting centre in the medulla. After injection of the poison into the stomach whatever the dose a definite interval elapses before vomiting takes place. It occurs at the same time as the other changes and it is never excessive. Even after vomiting death takes place thus showing that sufficient poison is absorbed before vomiting occurs.

From the facts that the animal remains conscious and that the reflexes are present until the respiratory centre is greatly affected, it follows that the poison has no previous effect on other parts of the brain and spinal cord. The animals do become sleepy but that can be explained by the muscular weakness and the fall of the blood pressure. The unconsciousness and absence of reflexes come on during the asphyxia.

It has already been mentioned that cases of abortion with fatal hæmorrhage have occurred by the use of this poison. The poison does not act directly on the uterus since it weakens involuntary muscle. The abortion must be due to the asphyxia produced by the poison. The uterine hæmorrhage is due to the dilatation of the blood vessels.

The poison is very virulent since the extract from only 2 gm. (30 grains) of the root is sufficient to kill a large monkey. Cases of murder have not been reported probably because the would-be victim detects the presence of something to be avoided owing to the acrid taste and smell of a strong solution. Cases of suicide have been reported. A few months ago a case of suspected "tuba" poisoning was recorded in Singapore. I am indebted to Dr. R. D. Keith acting Government Pathologist for the details. A quantity of "tuba" root was found in the room with the body. Post Mortem examination revealed nothing but venous congestion of the organs. The lungs possessed an acrid odour. Analysis of the stomach contents was negative. "Tuba" poison was not tested for, because chemical tests for this poison are unknown, although Greshoff (3) describes crystals of definite shape and colour, which are obtained from the poison.

The Post Mortem examinations in my experiments only show venous congestion of the organs.

It should be easy enough to detect the presence of "tuba" poison in the stomach contents by simply testing the effects, after boiling and filtering, of some of the fluid upon small fish, seeing that they are killed by very weak solutions of the poison.

The treatment indicated is that for poisons which produce muscular weakness and paralyse the respiratory centre. The natives of Sarawak administer sugar and cold baths. (8).

## Conclusions.

(1) "Tuba" fish-poison (*Derris elliptica*) causes death by paralysing the respiratory centre in the medulla. The sap from 2 gm. weight of the root when administered by mouth, is sufficient to kill a monkey (*Macacus nemestpinus*).

(2) It usually stimulates the respiratory centre before depressing it.

(3) It causes great weakness, because it weakens both voluntary and involuntary muscle and because it produces a great fall of blood pressure.

(4) It has no action upon the heart muscle or heart nervous mechanism.

(5) It produces a marked fall of blood pressure because it greatly weakens the muscle of the vessel walls, thus causing dilatation.

(6) It causes vomiting probably by stimulating the vomiting centre in the medulla; but, after swallowing large doses, sufficient poison to cause death is absorbed even if vomiting occurs.

(7) Fish poisoned by "tuba" can be eaten with impunity by man, because fish are killed by very small quantities of the poison.

(8) It is not antiseptic and its poisonous action is less marked on the lower members of the animal kingdom than on the more highly organised members. It kills mosquitœ larvæ and tadpoles but it is less toxic to these than to fish.

I am indebted to Dr. Hanitsch of The Raffles Museum and to Mr. I. H. Burkill of The Botanical Gardens, for assistance with the literature.

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(The author regrets that no reference has been made in the above paper to Van Hasselt's research published in the Archives internationales de Pharmacodynamie et Therapie, xxi. (1911) p. 243. Information regarding the existence of Van Hasselt's work was received after the paper had gone to press).

*Fig. 1. Monkey under chloroform anaesthesia.* Effects on respiration and blood pressure of injecting 1 c. c. 1 in 30 "tuba" poison into the femoral vein. A. Record of respiration. B. Blood pressure in femoral artery. C. Signal of injection. D. Time in two seconds. Note that the respiration and blood pressure are effected about forty seconds after the beginning of the injection. Respiration is greatly affected and ceases eleven minutes after the injection (Fig. I. X), thus producing death. The blood pressure falls very considerably at first but recovers in a few minutes, and is still well maintained when the respiration ceases. The heart was felt beating after the respiration ceased.

*Fig. 2. A. Monkey under chloroform anaesthesia, vagi intact.* Effects on respiration and blood pressure of injecting $\frac{1}{2}$ c. c. 1 in 100 "tuba" into the femoral vein. A. B. C. D. same readings as in fig. 1. Respiration is at first slightly augmented and later, weakened.

*Fig. 2. B. Same animal as in fig. 2 A, but with vagi cut.* Effects on respiration and blood pressure of injecting 1 c. c. 1 in 100 "tuba" into the femoral vein.

The respiration is similarly but more markedly affected in fig. 2 B, than in fig. 2 A, a larger dose of the poison being injected. The respiration recovers eventually. Note the fall of blood pressure in both cases. In fig. 2 A, the heart beats are just perceptible on the tracing. After cutting the vagi, Fig. 2 B, the heart beats are greatly augmented and the blood pressure is raised considerably, these results being the normal effects of cutting the vagi.

In this experiment death does not occur, because the dose of poison administered is non-lethal.